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SEED COLLECTION AND STORAGE FOR *ARCEUTHOBIUM* SPP.

by

ED F. WICKER

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SEED COLLECTION AND STORAGE FOR ARCEUTHOBIUM SPP.

Ed F. Wicker

INTERMOUNTAIN FOREST AND RANGE EXPERIMENT STATION
Forest Service
U.S. Department of Agriculture
Ogden, Utah
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SEED COLLECTION AND STORAGE FOR ARCEUTHOBIUM SPP.[1]

Ed F. Wicker

INTRODUCTION

Seeds of the six species and host forms of Arceuthobium used in these investigations mature in September and October, the exact time varying from year to year for any given location. Dissemination is via an explosive-fruit mechanism which is not completely understood (Johnson 1888; Heinricher 1915b; Hinds et al. 1963). If seeds are to be used to propagate the species, they should be mature when collected. This means "trapping" the seeds as they are forcibly ejected from the fruits. Previous investigations involving dwarf mistletoe seeds (Peirce 1905; Heinricher 1915a, 1915b, 1917; Hedgecock and Hunt 1917; Weir 1918; Palhinha 1942; Kuijt 1960) do not mention methods of seed collection and hence leave doubt as to seed maturity.

Frequently seeds cannot be sown immediately after collection; thus seed storage becomes necessary to insure a ready supply of suitable seeds.

LITERATURE REVIEW

When these investigations began, there were no documented techniques for collecting seeds of dwarf mistletoes. One report (Weir 1918) mentioned general conditions of seed storage. After these investigations were started, Scharpf and Parmeter (1962) reported on techniques that are advantageous in the collection, storage, and germination of seeds of A. campylopodum Engelm.

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SEED COLLECTION

Seeds of Arceuthobium were collected in September and October during the normal period of seed expulsion. Collections were made in dwarf mistletoe-infected coniferous stands in Washington and Idaho. Seed-producing female plants were bagged prior to seed expulsion. The expelled seeds adhered to the inner surface of the bags. Paper, cheesecloth, plastic, or sausage-casing bags were used depending upon the specific time of seed collection and the accessibility of the collection area.

The paper bag technique (Scharpf and Parmeter 1962) was used when seeds were actively being expelled. A paper bag was placed over the seed-bearing plant and the host branch was shaken to trigger seed expulsion. Some mature seeds were discharged when I placed the bags over the seed-producing plants.

Cheesecloth, plastic, or sausage-casing bags were used when seed collection was started before the period of maximum seed expulsion. Cheesecloth was wrapped around the seed-bearing plant and tied to the host branch. With plastic or sausage-casing bags, the seed-bearing plant and a portion of the host branch were enclosed in a bag and the bag was tied around the host branch with a piece of brightly colored plastic flagging, which also served as a locating marker. After the seed expulsion period, the host branch bearing the enclosed dwarf mistletoe plant was clipped and brought into the laboratory.

Because of the distances and time involved, it was possible to visit some of the seed collection areas only once. At some sites, if seeds were not yet expelled, infected host branches bearing seed-producing female dwarf mistletoe plants were clipped and brought to the laboratory. The cut ends of the branches were recut and placed in water. The flasks and branches were enclosed in bags and held until seeds were expelled.

SEED STORAGE

The principal goal of seed storage is to maintain the highest possible seed viability for prolonged periods. Starting with the meager data reported by Weir (1918), four seed storage methods were tested in the laboratory: (1) stratification, (2) dry cold storage, (3) moist cold storage, and (4) storage at room temperature (Anon. 1948). Storage in the field was at ambient air temperatures.

LABORATORY STORAGE

MATERIALS AND METHODS

Storage by stratification.--Sand obtained from the Snake River was washed in 2 percent HCl, rinsed twice with distilled water, and then sterilized with dry heat at 185° C. for 72 hours. The sterilized sand was moistened with sterile, de-ionized water and placed in sterilized petri dishes (65 X 100 mm.) to a depth of 30 mm. A sterile cheesecloth disc, cut to the diameter of the petri dish, was placed over the surface of the sand. Freshly collected, untreated, air-dried, dwarf mistletoe seeds were divided into lots of 100 seeds and placed upon the cheesecloth discs, one lot of seeds per dish. The seeds were scattered to avoid aggregation which would be conducive to the spread of molds. The seeds were then covered by another sterile cheesecloth disc which in turn was covered by 30 mm. of moist sand. The petri dishes were not covered. The stratified seeds were then refrigerated in the dark at 4-5° C. The relative humidity ranged from 30-45 percent as determined by a hygrothermograph placed in the room. Seeds of Arceuthobium douglasii Engelm. (1300),[2] A. campylopodum Engelm. f. laricis (Piper) Gill (4200), A. c. f. abietinum (Engelm.) Gill (400), and A. c. f. tsugensis (Rosend.) Gill (400) were stored by this method.

Dry cold storage.--Naturally expelled seeds (307) of A. douglasii were individually collected from the branches of Douglas-fir trees in the Thatuna Mountain Range, 3 miles east of Viola, Idaho, during December 1959. These seeds were placed dry and without any medium in an open petri dish and stored in a dark refrigerator at 5° C. Other use of the refrigerator exposed the seeds to some light during storage.

Freshly collected, untreated seeds of Arceuthobium douglasii (1000), A. americanum Nutt. ex. Engelm. (2400), A. campylopodum f. laricis (6000), A. c. f. campylopodum (Engelm.) Gill (400), A. c. f. abietinum (1400), and A. c. f. tsugensis (1200) were air dried (0-25° C.) for 1-4 days, counted into lots of 100 and scattered in open petri plates (15 X 90 mm.). No storage medium was used. The seeds were refrigerated in the dark at 4-5° C. and 35-45 percent relative humidity. The air in the refrigerator room was recycled at 5-minute intervals.

Moist cold storage.--Untreated seeds of Arceuthobium campylopodum f. campylopodum (1000) and A. c. f. laricis (1000) were air dried for 24-48 hours and then divided into lots of 100 seeds. Five lots of seeds of each host form were scattered in sterilized petri plates containing three discs of sterile Whatman #4 filter paper[3] (9.0 cm.) saturated with sterile distilled water. The plates were covered and stored in a dark refrigerator set at 5° C.

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[2] Number of seeds.

[3] Use of trade names in this paper does not necessarily imply endorsement by the U.S. Forest Service.

The other five seed lots of each host form were placed in sterile distilled water for 2 hours and then placed in sterilized, open petri plates. The external viscin had absorbed considerable water and the seeds were very slimy. These seeds were stored in the same refrigerator as the other lots.

Storage at room temperature.--Untreated seeds of Arceuthobium americanum (1700), A. douglasii (500), A. campylopodum f. campylopodum (1200), A. c. f. laricis (2000), A. c. f. abietinum (300), and A. c. f. tsugensis (700) were left in the bags. The seeds were not counted into lots. The tops of the bags were propped open and the bags were stored in the laboratory. The room temperature was 22-23° C.; the relative humidity, 8-30 percent. No attempt was made to exclude light.

RESULTS

Storage by stratification.--Seeds were first examined after 45 days in storage. All seeds were moldy and shriveled. Examination of cut seeds under a dissecting scope indicated that deterioration was well advanced and that the seeds were no longer viable. The embryo and endosperm were withered and shrunken to less than one-third their normal size and were dark brown to black in contrast to their normal green color.

After 60 days' storage, seed deterioration and development of molds were further advanced. The experiment was abandoned. Samples of the molded seeds were kept for isolation of the molds.

Dry cold storage.--The naturally expelled seeds collected from the branches of Douglas-fir during December 1959, were observed to be germinating on March 3, 1960. By March 20, 257 or 83.7 percent of these seeds were germinating. These seeds were later used in artificial inoculations in the greenhouse.

Examination after 30 days of the other lots of seeds stored in this manner revealed little change in external appearance except that the external viscin was dried and appressed to the seeds. Cut seeds showed only a slight shrinkage of the embryo and endosperm. The color of these tissues remained a normal green. Only an occasional seed (ca. 2 percent of the sample) was molded, but no seeds had germinated.

After 60 days there was little change in physical appearance. The embryo and endosperm retained their normal green color. One lot (100 seeds) of each species and host form was tested for viability using 2,3,5-triphenyl tetrazolium chloride (Flemion and Poole 1948; Lakon 1949, 1954; Smith 1951) at a concentration of 100 mg./100 ml. of water. The test lasted 3 days in darkness at room temperature. Results are shown in table 1.

Table 1.--Seed viability as determined by 2, 3, 5-triphenyl tetrazolium chloride after 60 days of dry storage at 5° C.

Dwarf mistletoe	Viability
	Percent
A. americanum	76
A. campylopodum f. abietinum	90
A. c. f. campylopodum	92
A. c. f. laricis	94
A. c. f. tsugensis	87
A. douglasii	91

The remainder of the dwarf mistletoe seeds from dry cold storage were subsequently used successfully to inoculate coniferous seedlings in the greenhouse and for germination tests.

Moist cold storage.--After 30 days' storage, the sample lots of seeds were supporting luxuriant mold growth. Cut seeds exhibited deterioration of the embryo and endosperm. The experiment was abandoned, but the molded seeds were kept for isolation of the molds.

Storage at room temperature.--In physical appearance, seeds examined after 30 to 60 days' storage were comparable to seeds in dry cold storage. After 30 days, the embryo and endosperm showed some shrinkage but were normal color. After 60 days, the color was a paler green. Some mold was present but no more than in seeds in dry cold storage.

Viability tests on samples after 60 days' storage gave surprising results. All seeds reacted negatively to tests using the TTC technique. A second sample was tested for viability after 67 days of storage and again the results were negative. The experiment was abandoned.

FIELD STORAGE

MATERIALS AND METHODS

Freshly collected seeds, seeds air dried for 24-48 hours in the field, and seeds air dried in the laboratory for 15 days at 22-23° C. were used in field storage experiments. All air-dried seeds were soaked in distilled water for 1-2 hours prior to field storage so they would adhere to the storage substrate. The storage substrates were 8-inch squares of cheesecloth several layers thick, glass bell jars, a wooden box, sausage-casing bags, and plastic bags.

Freshly collected seeds for field storage were obtained by bagging seed-bearing female dwarf mistletoe plants with sausage-casing and plastic bags. The plants were bagged in September and October. The bags were left until April when they were collected and brought into the laboratory for examination. Variable numbers of seeds of A. americanum, A. campylopodum f. campylopodum, A. c. f. laricis, and A. douglasii were stored by this method.

Two lots of 100 seeds each of A. douglasii, A. campylopodum f. campylopodum, and A. c. f. laricis were prepared for field storage on cheesecloth squares. These seeds were dried for 24-48 hours after collection and before field storage. They were then moistened to rehydrate the viscin so they would adhere to the cheesecloth. The seeds were stored during October in a mugo pine (Pinus mugo Turra) near the plant pathology greenhouses on the Washington State University campus. They were not examined until April. A laboratory check was conducted concurrently with this field-storage method, storing 100 seeds of each dwarf mistletoe by the dry cold storage method.

Three lots of 100 seeds each of A. douglasii, A. campylopodum f. laricis, A. c. f. abietinum, and A. c. f. tsugensis were air dried for 15 days at 22-23° C. and then affixed to cheesecloth by rehydration of the viscin. The cheesecloth squares were fastened to the bole of Douglas-fir trees at a storage site in the Thatuna Mountain Range (R. 5 W., T. 40 N., Sec. 5 - Boise Meridian).

Following 15 days' drying at 22-23° C., lots of 100 seeds each of A. douglasii, A. campylopodum f. campylopodum, and A. c. f. laricis were attached to the inner surfaces of glass bell jars. Comparable lots were attached to the inner surfaces of a wooden box. The glass jars and wooden box were hung over branch stubs in Douglas-fir trees in the Thatuna Mountain Range. The storage period was late October to late April. A hygrothermograph and weather instrument shelter were installed at the site in November to provide a continuous record of the temperature and relative humidity.

RESULTS

The germinability of all field-stored seeds was tested in April by the hydrogen peroxide (H_2O_2) technique (Wicker 1962). Germination was at 10° C. in the dark. All seeds that had been air dried in the laboratory for 15 days before field storage failed to germinate, regardless of the storage substrate employed. Seeds stored on cheesecloth squares and in the wooden box had molded, but there was no mold development on those in glass jars. Of interest, naturally expelled seeds of A. douglasii on the branches and foliage of Douglas-fir trees at the storage site were germinating in April.

Of the seeds which were air dried for 24-48 hours and then stored in the field on cheesecloth, 55-82 percent had germinated by April (table 2). Although none of the laboratory check seeds had germinated, the H_2O_2 test demonstrated their germinability (table 2, lots #3). There was no visible evidence of molds on seeds which had germinated but some could be seen on seeds which did not germinate. Seeds which had not

Table 2.--Germination of air-dried[1] dwarf mistletoe seeds (100 seeds/lot) after field storage for 6 months on cheesecloth

Dwarf mistletoe	Lot no.	Germination	Av. germination
		Percent	
A. c. f. campylopodum	1	77	79.5
	2	82	
	[2][3]	[3]84	
A. c. f. laricis	1	62	65.5
	2	69	
	[2][3]	[3]56	
A. douglasii	1	63	59.0
	2	55	
	[2][3]	[3]54	

[1] 24-48 hours at "field temperatures."
[2] Laboratory storage, 5° C. and 35-45 percent relative humidity.
[3] Determined by H_2O_2 treatment.

germinated by the end of the field-storage period were treated with H_2O_2 and an additional 2 percent of A. douglasii, and 5 percent of A. c. f. laricis and A. c. f. campylopodum subsequently germinated.

All seeds stored over winter in the plastic bags had deteriorated by the end of the storage period. All the bags had collected water from the winter rains and snows, transpiration, and condensation. Consequently, most of the seeds had rotted. Even the few seeds which remained above the water in the bags were molded. No seeds were suitable for viability or germination tests.

Seeds overwintered in the sausage-casing bags were in much better condition than the seeds stored in plastic bags. While some seeds had molded, the majority appeared to be in good condition. Only seeds of A. c. f. laricis showed any germination at the end of the storage period and those germinating were intermingled with dead host needles which had been cast in the bags. Seeds remaining stuck to the inner surfaces of the bags were not germinating. Germinability (H_2O_2 treatment) of seeds stored by this method is shown in table 3.

Table 3.--Germination of dwarf mistletoe seeds after 6 months' field storage in sausage-casing bags

Dwarf mistletoe	No. seeds stored	No. seeds germinated (H_2O_2)	Germinability	No. bags
			Percent	
A. americanum	571	468	82	5
A. campylopodum f. campylopodum	143	93	65	2
A. c. f. laricis	476	[1]281	59	5
A. douglasii	237	97	41	7

[1]Includes seeds naturally germinating.

DISCUSSION

The systemic nature of the infections and the distribution of the shoots produced by A. douglasii make collection of large seed lots difficult. This was the limiting factor in the investigations reported for the species. The localized infections of the other five taxa are much more conducive to collection of large quantities of seed.

The most satisfactory traps for collecting seeds are sausage-casing bags. The bags are porous and permit gaseous and aqueous exchange with the atmosphere. Female infections can be bagged several months prior to seed maturity and the seeds can be left in the bags several months after expulsion without appreciable damage. Seeds may be stored in the laboratory or in the field in these bags with a minimum of molding. The bags are costly compared with paper bags, plastic bags, or cheesecloth. But they are durable. Some have been used for 3 consecutive years and are still serviceable. Because of their durability, I have used sausage-casing bags almost exclusively for seed collection.

Use of paper and plastic bags for seed collection had not been reported prior to the present investigations, although Scharpf and Parmeter (1962) later reported on the use of paper bags. While at the collection site, I used paper bags to collect seeds then being expelled. After these had been collected, each infection was enclosed in a plastic bag to trap the remaining seeds. The plastic bag was retrieved on the next visit. These "traps" are low in cost and the seeds can be stored in the paper bags in the laboratory.

There are serious limitations to paper bags, however. They cannot be used when the host's foliage is wet. Yet, wet periods are ideal for seed collecting because turgidity is a factor in the explosive-fruit mechanism. Even during dry weather, the paper bags absorb moisture from the seeds. They also are easily torn by tree branches and shrubs. Considerable seed loss may result because all seeds do not stick to the inner surface of the bag.

The major disadvantage of plastic bags is their nonporous nature. Free water accumulates inside the bags and provides conditions highly conducive to seed deterioration and molding. Plastic bags are unsatisfactory for storing seeds because of these factors.

Cheesecloth is less convenient to use than either paper or plastic bags, but it does eliminate serious moisture and mold problems. Seeds can be stored on the cheesecloth in the laboratory or the field. The major disadvantage is getting the seeds off the cheesecloth. Some can be washed off with water. The remainder must be picked off with forceps.

My interpretation of the conditions reported by Weir (1918) as suitable for dwarf mistletoe seed storage resulted in failure. Stratification at low temperatures would seem a practical way to maintain desirable storage conditions, but seeds stored by this method deteriorated as a result of luxuriant growth of mold fungi. Perhaps this method of seed storage would be satisfactory if completely aseptic conditions could be established and maintained. This proved impossible by any of the techniques tried. The old adage "one rotten apple will spoil the entire barrel" seems appropriate here.

Successful seed storage was achieved under dry, cold conditions. The first indications that dwarf mistletoe seeds could be stored by this method were obtained with seeds of A. douglasii collected from branches of Douglas-fir in December 1959. They not only remained viable when stored by this method, but 83.7 percent of the seeds began germinating while still in artificial storage and without supplemental treatment. This was the only time that naturally disseminated seeds germinated while in laboratory storage without supplemental treatment. However, this was also the only time that seeds were collected 3 months after dissemination and then stored in the laboratory. In subsequent experiments an extract of Douglas-fir bark did not stimulate germination of freshly collected seeds. Seeds overwintered in the field on cheesecloth or in sausage-casing bags (tables 2 and 3) germinated without stimulation. Therefore, no special stimulus seems necessary for seed germination. Satisfactory germination after storage is dependent on moisture content, oxygen, and temperature conditions prior to and during storage. Extreme variation may exist for these requirements between plant species (Owen 1956; Crocker and Barton 1957; Kramer and Kozlowski 1960; Mayer and Poljakoff-Mayber 1963).

Dry, cold storage of freshly collected seeds, air dried for not more than 48 hours, was equally successful. Viability (TTC test) was high after 60 days in storage (table 1). Other tests of seeds stored by this method indicated high viability and germinability (Wicker 1962, 1965). Some seeds remained viable for more than 12 months. Molds are not a problem although some mold growth does occur.

Cold storage under moist conditions without stratification was unsuccessful. Molds destroyed the seeds. This method of storage might prove satisfactory under completely aseptic conditions but it is obviously impractical, particularly if large amounts of seeds need to be stored. Cool, moist conditions are suitable for seed germination, when used aseptically, as reported by Scharpf and Parmeter (1962). But these conditions are most difficult to maintain for prolonged storage periods.

Seed storage at room temperatures was not successful. Molds and high relative humidities were not the detrimental factors. The relative humidity was low (8-30 percent) and mold was very sparse. The detrimental factor of this method of storage was definitely shown to be high temperatures (Wicker 1965).

Dry, cold (5° C., 35-45 percent relative humidity, dark) laboratory storage more closely simulates the environmental conditions to which seeds are exposed during the fall and winter months in nature than do the other laboratory storage methods employed. Dry, cold storage provided adequate moisture conditions for retention of seed viability, but not enough moisture for luxuriant development of mold fungi. Moist cold storage and stratification provided a surplus of moisture and created conditions favorable for fungal development. Percentage germination of seeds following 6 months of dry, cold laboratory storage (Wicker 1965) and 6 months of field storage (tables 2 and 3) are comparable.

The efficacy of storage under partial vacuum (Anon. 1948), or at subfreezing temperatures (Isaac 1934; Curtis 1955; Jones 1962) was not evaluated for dwarf mistletoe seeds, although dry storage for 32 days at -3° C. followed by 145 days at 10° C. gave as good germination as storage at 10° C. continuously for 177 days (Wicker 1965). Thus, freezing does not kill the seeds within this period and at the temperature tested.

Cheesecloth and sausage-casing bags are both satisfactory for storing seeds in the field (tables 2 and 3). There was only minor mold development on freshly disseminated seeds. The sausage-casing bags are more convenient to use in the field and require less seed handling. Natural seed germination is low with this method. In only one of four taxa did natural germination occur during field storage in sausage-casing bags (table 3). Thus, storage in these bags provides more physiologically uniform seeds. With cheesecloth, natural germination is rather high (table 2). Both substrates are currently being used, but because of the resulting uniformity and the rapid germination obtained with H_2O_2 (Wicker 1962), the sausage-casing bag method is preferred.

Successful storage of dwarf mistletoe seeds depends on seed treatment prior to storage as well as on the method of storage. The effect of prolonged high temperature was obvious from comparing seeds air dried for 24-48 hours (table 2) vs. air dried for 15 days at 22-23° C. before field storage on cheesecloth.

Storage methods which prevent a gaseous exchange with the atmosphere and which permit free water to accumulate in the presence of fungal and bacterial contaminants are unsatisfactory. The use of nonporous plastic bags was the only completely unsatisfactory field storage method employed. The suitability of wood as a field storage substrate was not adequately tested. That wood can be a satisfactory substrate for field storage is supported by the observed germination of naturally implaced seed on weathered fence posts (Peirce 1905; personal observations). The glass bell jars did not absorb or retain moisture, and very little fungal development occurred on seeds affixed thereto.

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